Thus a single channel confocal scanner has been used to measure a number of sample sites but only sequentially and not simultaneously, and such a system is not appropriate for high throughput scanning where simultaneous detection and assessment of a large number of sites is required, such as in a 96 well microtitre plate or higher density format array or array of microcapillaries.

Summary of the invention

According to one aspect of the present invention, a plurality (N) of single channel confocal optical systems and photoelectric detectors or detecting areas are arranged in parallel to form a plurality of reading heads arranged side-by-side so as simultaneously to read a corresponding plurality of adjacent sites.

The sites may be in a column or in an array such as a microtitre plate.

The optical systems may be arranged in a single line for reading column by column of a multi-column array, or in a staggered pattern for simultaneously viewing sites in different columns if this were preferred.

This system would comprise essentially (N) independent confocal systems, each with its optic axis aligned with one sample site.

Advantageously only one laser source is provided which may be split into an appropriate plurality of beams, conveyed each by a fibre optic cable to individual sample sites.

The light emitted from the separate sites may be conveyed to individual detectors, or discrete regions of an array detector, via optical fibres.

Preferably the reading heads are independently adjustable so

each can be positioned accurately over or under a sample. To this end a special opto-mechanical device may be provided to bring each scanner's optic axis into alignment by means of an appropriate scan in the Y-direction.

Where there is a tolerance in the position of the samples, such as in the case of capillaries, which result in uncertainty in the Y-direction, adjustment is preferably provided.

Such an arrangement could be used to inspect say 8 or 12 sites at a time, but an arrangement to inspect larger numbers simultaneously could be costly, complex, and cumbersome. We would refer to such arrangements as "ganged" systems.

According to a more preferred aspect of the invention in a method of imaging a plurality of micro-sample light emitting sites simultaneously onto separately addressable detectors, which may simply be discrete regions of the array detector, so that light emitted from each site can be monitored by one of the detectors, a corresponding plurality of objective lenses are located adjacent to the micro-sample array with one objective lens for each micro-sample, the latter are located at or near the focal point of each of the lenses so that light emanating from each micro-sample is collected by its respective objective lens and converted into a beam of parallel or near parallel rays, the objective lenses are arranged so that the axes of all the beams issuing therefrom are parallel and spaced apart, the beams are focused by a focusing lens through a single point and collected beyond that point by detector lens means which serves to reconstitute the parallel beams for presentation to the detector array.

If near parallel the rays may be converging or diverging and the optical system downstream from the objective is adapted accordingly.

The axes of the objectives may be angled if desired with

relative to the array of objective lenses in manner known per se so as to present groups of 96 wells making up the 384 wells, to the 96 lens array. Thus a 2 x 2 movement of the XY stage would be required to complete the inspection of all of the well sites in the 384 well array.

According to another preferred feature of the invention, the parallel beams of light directed towards the detector array may be transferred to the latter via optical fibres, preferably in the form of a fibre optic bundle or fibre optic plate.

According to a further preferred feature of the invention, where a bundle of fibres is employed, the arrangement of the fibres in the bundle may differ between the input and output ends thereof so that the array of fibres which convey the light paths to the different regions of the detector array conforms more to the shape of the detector array in the XY plane and enables for example, optimal utilisation of a generally square array by the rectangular proportions of an 8 x 12 micro-sample array.

The detector array may typically be a charge coupled device having a large number of separately addressable regions (each of which is commonly referred to as a pixel) and groups of adjacent pixels (or individual pixels) from the detector for each sample are addressed, to enable good resolution to be obtained in the XY sense as between the light from one sample and another.

The charge coupled device may be cooled, for example cryogenically so as to reduce background noise signals associated therewith.

According to a further feature of the invention, the detectors comprise an array of photomultipliers, one photomultiplier for each of the channels (optical paths).

Thus if there are 96 channels, 96 photomultipliers will be required.

Preferably miniature photomultipliers are employed, and where sufficiently small photomultipliers are unavailable, optical fibres or bundles forming cables may be employed to convey the light from each of the apertures in the mask such as is shown in Figure 4 or 5 of the accompanying drawings, to the 96 windows of 96 photomultipliers which together occupy an area considerably greater than that of the mask.

Alternatively a larger detector lens is employed so that the 96 channels are spread over a larger area.

As previously mentioned, the CCD array may of course be cryogenically or otherwise cooled for the sample purpose, the use of photomultipliers obviates the need for cryogenic cooling.

According to another feature of the invention, the photomultipliers are replaced by an image intensifier or an intensified CCD.

The use of a more sensitive detector such as the use of photomultiplier tubes, an image intensifier or an intensifier CCD, enables time resolved fluorescence of luminescence application to be detected.

In particular the use of a more sensitive detector enables time resolved assay analysis to be performed which involves photo-exciting a fluorophor with a pulsed source of radiation for example a pulsed laser, during which time the detector is gated off so as not to be responsive to any of the excitation radiation and the fluorophor in the assay can be investigated by gating on the detector after a suitable time delay, typically of the order of picoseconds or microseconds, and thereafter reading out the charge pattern in the detector.

Time resolved applications such as described require detectors which can be gated on and off and photomultiplier tubes, image intensifiers and intensified CCD arrangements can be gated in this way electronically so as to enable the delays and short integration periods to be generated as required by time resolved fluorescence or luminescence applications.

In this connection, it is to be understood that the present invention is not limited to fluorescence applications but includes luminescence applications.

Thus the invention is not limited to the nature of the detector or to the technique in which the different regions of the detector are addressed to enable light emanating from one sample to be distinguished from light emanating from another, nor is the invention limited to any of the circuits or computing techniques which may be employed for processing electrical signals obtained by addressing the different regions of the detector array.

Further lenses for focusing the parallel beams of light directed towards the detector array may be employed, for improving the resolution at the detector surface either in combination with a fibre optic transfer bundle, or otherwise.

Since the spacing between, and the actual size of, the sample sites in a micro-sample array, are very small, and can be of the order of microns, micro lenses, optionally in combination with a fibre optic transfer plate may be employed in the objective lenses adjacent the micro-samples.

In essence the micro-sample objective lenses are equivalent to microscope objectives in that they are short focal length lenses designed to form an image of a tiny object (in this case light being emitted from a small region in a micro-sample). However as distinct from a conventional microscope objective, the optical characteristic of the sample objectives is such as

to produce a parallel beam of light therefrom so that in this sense the objective can be said to have one infinite conjugate.

By utilising high quality components and projecting the excitation radiation to the samples via the objective lenses, the excitation radiation can be focused to precisely the regions of interest in the samples, to reduce excitation of surrounding sample material.

Where a filter is located ahead of the detector, apertured masks may be placed on either side of the filter to collimate the parallel beam to further reduce background and cross-talk.

The invention also lies in apparatus adapted to perform the above methods and comprising means for supporting a micro-sample array on a substrate in close proximity but parallel to an array of micro lenses arranged so as to correspond on a one to one basis with the positions and spacing of at least some of the micro-samples on the substrate, each of the micro lenses being positioned relative to a region of its related micro-sample by a distance equal to the focal length of the lens so that light emitted from that region of the micro-sample will emerge from the lens as a parallel beam parallel to the axis of the lens, and the parallel beams of light are focused by means of a single focusing lens onto a detector lens so as to produce an image of the micro-sample light emissions in the plane of an array of individually addressable photoelectric detectors, such as regions of an addressable CCD array, and circuit means is provided to which signals read out from the array are supplied in the form of a sequence of digital values or otherwise, each corresponding to the light incident on a region of the detector for a given period of time from one of the micro-samples, and computing and analysing circuit means is provided, responsive to the electrical signals, together with memory means for storing data indicative of the light found to be emitted from each of the micro-samples, for storing those values together with address information, whereby each

stored value can be identified with the micro-sample on the substrate from which the light giving that value has been emitted by reference to the position of the region in the detector array and by correlating the position of the sample in the sample array.

The apparatus may further include a beam splitter, such as a dichroic mirror, interposed in the optical path between the micro lenses and the focusing lens to enable on the one hand light to pass from the lenses to the focusing lens, and on the other hand to enable excitation radiation, typically light of a particular wavelength, to be reflected as a parallel beam towards the micro lenses, thereby utilising the optical focusing characteristics of the micro lenses to focus the parallel light into spots of light which register with the micro-samples so that the latter are individually radiated by excitation light which is predominantly incident on that region of each micro-sample which is to be inspected for fluorescence after the excitation radiation has been removed, and filter means may be provided in the optical path between the beam splitter and the detector array to generally attenuate any excitation wavelength radiation travelling towards the detector and generally prevent such radiation from reaching the detector.

The apparatus preferably includes a pinhole aperture at the focal point of the focusing lens so as to improve the on-axis resolution of the optical system and assist in attenuating unwanted fluorescence such as from background material and other components of an assay ahead of or behind the region of interest in a micro-sample measured along the optical axis of the objective, from reaching the detectors.

The apparatus typically includes a laser source, as the source of excitation radiation a beam expander for enlarging the cross-section of the laser beam and presenting a generally uniform parallel beam of excitation radiation for entry into

the imaging system via the beam splitter or dichroic mirror.

Shutter means may be provided to inhibit the passage of the laser light, except when required for excitation purposes, and further shutter means may be provided synchronised with that associated with the laser source to prevent light of any wavelength reaching the detector whilst excitation light is projected into the system.

The invention also comprises a method of analysing fluorescence emitted by radiation excited samples in an array of samples comprising the steps of focusing light emitted from each said sample at infinity so as to form a parallel beam, in parallel with the light from all of the other sample sites making up the array, subsequently focusing all the parallel light paths through a single point and locating at the point a small aperture to restrict unwanted light from fluorescing material upstream and downstream of the sites of interest in the samples, and re-establishing a parallel array of light beams by the use of a further lens so as to present to an addressable detector array a plurality of parallel light paths corresponding to the light paths from the samples, and individually addressing different regions of the detector array onto which the parallel light paths impinge, to determine the light incident thereon, and storing data relating to the quantity of incident light on each said region of the detector array together with address information to enable the data to be reconciled with the position of the sample in the array on the substrate to which that data relates.

The method typically further comprises the step of introducing periodically into the optical system excitation wavelength illumination and projecting same through the optical imaging devices associated with the array of samples to project the excitation illumination onto a specific region in each said sample, thereafter extinguishing the excitation wavelength light and enabling fluorescence caused by the excitation to

CLAIMS

1. A method of measurement of radiation, wherein a plurality (N) of single channel confocal optical systems and photoelectric detectors or detecting areas are arranged in parallel to form a plurality of reading heads arranged side-by-side so as simultaneously to read a corresponding plurality of adjacent sites emitting radiation.

2. A method according to claim 1, wherein the sites are arranged in a column or in an array such as a microtitre plate, and the optical systems are arranged in a single line for reading column by column of a multi-column array, or in a staggered pattern for simultaneously viewing sites in different columns.

3. A method according to claim 1 or claim 2, using (N) independent confocal systems, each with its optic axis aligned with one sample site.

4. A method according to any of claims 1 to 3, wherein light from a single laser source is split into an appropriate plurality of beams, conveyed each by a fibre optic cable to individual sample sites.

5. A method according to any of claims 1 to 4, wherein light emitted from the separate sites is conveyed to individual detectors, or discrete regions of an array detector, via optical fibres.

6. A method according to any one of claims 1 to 5, wherein the reading heads are independently adjustable so that each can be positioned accurately over or under a sample.

7. A method according to claim 6, wherein a special opto-

mechanical device is provided to bring each scanner's optic axis into alignment by means of an appropriate scan in the Y-direction.

8. A method of imaging a plurality of micro-sample light emitting sites simultaneously onto separately addressable detectors, which may simply be discrete regions of the array detector, so that light emitted from each site can be monitored by one of the detectors, wherein a corresponding plurality of objective lenses are located adjacent to the micro-sample array with one objective lens for each micro-sample, the latter are located at or near the focal point of each of the lenses so that light emanating from each micro-sample is collected by its respective objective lens and converted into a beam of parallel or near parallel rays, the objective lenses are arranged so that the axes of all the beams issuing therefrom are parallel and spaced apart, and the beams are focused by a focusing lens through a single point and collected beyond that point by detector lens means which serves to reconstitute the parallel beams for presentation to the detector array.

9. A method according to claim 8, wherein the axes of the objectives are angled with appropriate adjustment of the optical characteristics of the objectives and/or downstream optical system.

10. A method according to claim 8 or claim 9, wherein a single focusing lens (which may be a multi-component lens) is employed for directing all the beam paths through the single point.

11. A method according to any of claims 8 to 10, wherein optical discrimination between fluorescence emanating from upstream and downstream region is improved by placing a small aperture pinhole at the focal point of the focusing lens so that light which is not emanating from the focal point of each of the objective lenses adjacent the micro-sample sites will be out of focus at the small aperture pinhole.

12. A method according to any of claims 8 to 11, wherein, the micro-samples are positioned relative to the micro-sample objective lenses so that the region of interest is as close as possible to the focal point of the respective objective lens.

13. A method according to any of claims 8 to 12, wherein the samples are located on a planar support with the regions of interest all in the same plane so that the objective lenses can likewise all be in the same plane parallel to that containing the regions of interest in the samples.

14. A method according to any of claims 8 to 13, including the step of adjusting the position of the micro-sample array relative to the lens array and also the step of individually adjusting the position of at least the objective lenses relative to the micro-samples or vice versa to ensure that the regions of interest in the micro-samples are at the focal point of the respective micro-sample objective lenses.

15. A method according to any of claims 8 to 14, wherein in order to provide spectral separation based on wavelength, a filter is included in the light path either between the micro-sample objective lenses and the focusing lens means ahead of the pinhole, or between the detector lens and the detector array.

16. A method according to claim 15, wherein the spectral filter is located in a region in which the light paths are parallel or nearly parallel.

17. A method according to any of claims 8 to 16, according to which where fluorescence is the mechanism which generates the radiation which is to be focused onto a detector, excitation radiation to produce the fluorescence is applied only to a region of interest within each micro-sample rather than over the whole of the micro-sample.

18. A method according to claim 17, wherein excitation radiation is injected into the multipath optical system so as to proceed in a parallel sense towards the array of objective lenses, in an opposite sense to the light which emanates from the micro-samples, so as to be focused by the objective lenses onto the region of interest in each micro-sample.

19. A method according to claim 18, wherein the excitation radiation is injected as a parallel beam into the optical path, at right angles thereto, and a 45° beam splitting device is provided onto which the parallel excitation radiation is incident and from which it is directed in a parallel manner towards the micro-sample imaging lenses, but through which radiation from the micro-samples can pass to the focusing lens.

21. A method according to any of claims 8 to 19, wherein excitation radiation is produced using a laser such as an argon ion laser, and a beam expander is employed to expand the cross-section of the laser beam into a relatively large area beam equivalent to the area of the parallel array of micro-sample objective lenses.

21. A method according to any of claims 8 to 20, having an 8 x 12 array of micro-sample objective lenses on the same 8 x 12 matrix as a standard 96 well plate, and if the imaging system is to be used to inspect for example a 384 well plate, the latter is mounted on an XY stage so that it can be moved relative to the array of objective lenses in manner known per se so as to present groups of 96 wells making up the 384 wells, to the 96 lens array.

22. A method according to any of claims 8 to 21, wherein the parallel beams of light directed towards the detector array are transferred to the said array via optical fibres, in the form of a fibre optic bundle or fibre optic plate.

23. A method according to claim 22, wherein where a bundle of

fibres is employed, the arrangement of the fibres in the bundle differs between the input and output ends thereof so that the array of fibres which convey the light paths to the different regions of the detector array conforms more to the shape of the detector array in the XY plane.

24. A method according to any of claims 8 to 23, wherein the detector array is a charge coupled device having a large number of separately addressable regions (each of which is commonly referred to as a pixel) and groups of adjacent pixels (or individual pixels) from the detector for each sample are addressed, to enable good resolution to be obtained in the XY sense as between the light from one sample and another.

25. A method according to claim 24, wherein the charge coupled device is cooled, for example cryogenically.

26. A method according to any of claims 8 to 23, wherein the detector array comprises an array of photomultipliers, one photomultiplier for each of the channels (optical paths).

27. A method according to claim 26, wherein optical fibres or bundles forming cables are employed to convey the light from each of the apertures in a mask to the windows of photo-multipliers which together occupy an area considerably greater than that of the mask.

28. A method according to claim 26 or 27, wherein the photomultipliers are replaced by an image intensifier or an intensified CCD.

29. A method according to any of claims 26 to 28, wherein the photomultiplier tubes, image intensifiers and intensified CCD arrangements are gated electronically so as to enable the delays and short integration periods to be generated as required by time resolved fluorescence or luminescence applications.

30. A method according to any of claims 8 to 29, wherein further lenses for focusing the parallel beams of light directed towards the detector array are employed to improve resolution at the detector surface either in combination with a fibre optic transfer bundle, or otherwise.

31. A method according to any of claims 8 to 30, wherein micro lenses, optionally in combination with a fibre optic transfer plate are employed in the objective lenses adjacent the micro-samples.

32. A method according to claim 31, in which micro lenses have one infinite conjugate.

33. A method according to any of claims 8 to 32, wherein where a filter is located ahead of the detector, apertured masks are placed on either side of the filter to collimate the parallel beam to further reduce background and cross-talk.

34. Apparatus adapted for performing the method of any of claims 1 to 33, comprising means for supporting a micro-sample array on a substrate in close proximity but parallel to an array of micro lenses arranged so as to correspond on a one to one basis with the positions and spacing of at least some of the micro-samples on the substrate, each of the micro lenses being positioned relative to a region of its related micro-sample by a distance equal to the focal length of the lens so that light emitted from that region of the micro-sample will emerge from the lens as a parallel beam parallel to the axis of the lens, and the parallel beams of light are focused by means of a single focusing lens onto a detector lens so as to produce an image of the micro-sample light emissions in the plane of an array of individually addressable photoelectric detectors, such as regions of an addressable CCD array, and circuit means is provided to which signals read out from the array are supplied in the form of a sequence of digital values or otherwise, each corresponding to the light incident on a

region of the detector for a given period of time from one of the micro-samples, and computing and analysing circuit means is provided, responsive to the electrical signals, together with memory means for storing data indicative of the light found to be emitted from each of the micro-samples, for storing those values together with address information, whereby each stored value can be identified with the micro-sample on the substrate from which the light giving that value has been emitted by reference to the position of the region in the detector array and by correlating the position of the sample in the sample array.

35. Apparatus according to claim 34, further including a beam splitter, such as a dichroic mirror, interposed in the optical path between the micro lenses and the focusing lens to enable on the one hand light to pass from the lenses to the focusing lens, and on the other hand to enable excitation radiation, typically light of a particular wavelength, to be reflected as a parallel beam towards the micro lenses, thereby utilising the optical focusing characteristics of the micro lenses to focus the parallel light into spots of light which register with the micro-samples so that the latter are individually radiated by excitation light which is predominantly incident on that region of each micro-sample which is to be inspected for fluorescence after the excitation radiation has been removed, and filter means provided in the optical path between the beam splitter and the detector array to generally attenuate any excitation wavelength radiation travelling towards the detector and generally prevent such radiation from reaching the detector.

36. Apparatus according to claim 34 or claim 35, including a pinhole aperture at the focal point of the focusing lens so as to improve the on-axis resolution of the optical system and assist in attenuating unwanted fluorescence such as from background material and other components of an assay ahead of or behind the region of interest in a micro-sample measured along the optical axis of the objective, from reaching the

detectors.

37. Apparatus according to any of claims 34 to 36, including a laser source as the source of excitation radiation a beam expander for enlarging the cross-section of the laser beam and presenting a generally uniform parallel beam of excitation radiation for entry into the imaging system via the beam splitter or dichroic mirror.

38. Apparatus according to any of claims 34 to 37, including shutter means to inhibit the passage of the source light, except when required for excitation purposes, and further shutter means synchronised with that associated with the source to prevent light of any wavelength reaching the detector whilst excitation light is projected into the system.

39. A method of analysing fluorescence emitted by radiation excited samples in an array of samples comprising the steps of focusing light emitted from each said sample at infinity so as to form a parallel beam, in parallel with the light from all of the other sample sites making up the array, subsequently focusing all the parallel light paths through a single point and locating at the point a small aperture to restrict unwanted light from fluorescing material upstream and downstream of the sites of interest in the samples, and re-establishing a parallel array of light beams by the use of a further lens so as to present to an addressable detector array a plurality of parallel light paths corresponding to the light paths from the samples, and individually addressing different regions of the detector array onto which the parallel light paths impinge, to determine the light incident thereon, and storing data relating to the quantity of incident light on each said region of the detector array together with address information to enable the data to be reconciled with the position of the sample in the array on the substrate to which that data relates.

40. The method according to claim 39, further comprising the

step of introducing periodically into the optical system excitation wavelength illumination and projecting same through the optical imaging devices associated with the array of samples to project the excitation illumination onto a specific region in each said sample, thereafter extinguishing the excitation wavelength light and enabling fluorescence caused by the excitation to pass through the same optical devices to emerge as parallel rays of light for transfer to a detector, for analysis as above mentioned.

41. The method according to claim 39 or claim 40, wherein the objectives are arranged above or below a sample array.

42. The method according to any of claims 39 to 41, when used to perform immediate fluorescence analysis or time resolved fluorescence analysis in which a shutter is provided to inhibit the transfer of light to the detector, excitation radiation is supplied for a short interval of time and then shut off (either by pulsing the source out using further shutter or both), after a selected interval of time the shutter preventing transfer of light to the detector is opened and after an appropriate integration interval (which can be very short or longer as described) the residual charge pattern on the charge coupled detector array is interrogated to generate a signal relating to the charge pattern (and therefore indicative of light incident thereon) for processing and storage as aforesaid.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
KWN/C1124.01/C		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/01576	20/04/2000	26/06/1999
Applicant		
CAMBRIDGE IMAGING LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   MICROPLATE READER

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
C1124.01/C		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01576	20/04/2000	26/06/1999
International Patent Classification (IPC) or national classification and IPC G01N21/25		
Applicant PACKARD INSTRUMENT COMPANY, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 17 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/01/2001	07.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Huenges, A Telephone No. +49 89 2399 2280

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01576

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originaliy filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-5,8-10,17-24	as originally filed			
6,7,7a,11-16, 16a	as received on	05/07/2001	with letter of	28/06/2001

Claims, No.:

1-32	as received on	05/07/2001	with letter of	28/06/2001

Drawings, sheets:

1/6-6/6	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-32
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-32
Industrial applicability (IA)	Yes:	Claims	1-32
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: DE 197 48 211 A (CARL ZEISS) 6 May 1999 (1999-05-06)
D2: H.J. TIZIANI ET AL.: 'Three-dimensional analysis by a microlens-array confocal arrangement' APPLIED OPTICS., vol. 33, no. 4, 1 February 1994 (1994-02-01), pages 567-572, XP000429123 OPTICAL SOCIETY OF AMERICA,WASHINGTON., US ISSN: 0003-6935
D3: GB-A-2 315 131 (CAMBRIDGE IMAGING) 21 January 1998 (1998-01-21)

2. **Inventive step**

2.1. Independent **claims 1, 25 and 29**

The subject-matter of **claims 1, 25 and 29** is new (Article 33(2) PCT) but not inventive (Article 33(3) PCT).

Regarding **claim 1** document **D1** discloses a method of imaging a plurality of micro-sample light emitting sites simultaneously onto separately addressable detectors (col. 2, ll. 25-30) wherein a plurality of objective lenses (2i, fig. 2) are located adjacent to the micro-sample array (see micro titre plate (1), fig. 2) with one objective lens for each micro-sample (col. 3, ll. 19-21), the latter are located at or near the focal point of each of the lenses (the position of the micro-sample array in fig. 2 is necessarily at the focal point of the micro-sample array as the light beam between the micro-lens array (2i) and the focussing lens (41) is parallel and as the beam focuses to a single point at the position of the lens (5), fig. 2) and the beams are focused by a focusing lens through a single point (at the position of lens (5), fig. 2) and collected beyond that point by detector lens means (42, fig. 2) which serves to reconstitute the parallel beams (see fig. 2) for presentation to the detector array (6, fig. 2).
The subject-matter of claim 1 differs therefrom in that a pinhole aperture is located at the focusing point of the focussing lens.

The problem to be solved by the present invention may therefore be regarded as the detection of light solely emanating from the focal point of each of the objective lenses.
However, a plate with a small aperture for detection of light emanating from the focal point of each of the objective lenses of a microlens array has already been used in a similar method, see document D2, figure 4 (aperture (2)). It would be obvious to the person skilled in the art, namely when the same result is to be achieved, to apply this feature to document D1, thereby arriving at a method according to claim 29.

Regarding **claim 25**, document **D1**, which is considered to represent the most relevant state of the art, discloses an apparatus adapted for performing the method of claims 1 or 8, comprising means for supporting a micro-sample array on a substrate (means for supporting the micro-titre plate (1, fig. 2) are implicitly disclosed) in close proximity but parallel to an array of micro lenses (2i, fig. 2) arranged so as to correspond on a one to one basis with the positions and spacing of at least some of the micro-samples, the micro-samples being positioned at the focal points of their related micro lenses (implicitly disclosed with parallel rays between micro lenses (2i) and lens (41), fig. 2), and the parallel beams of light are focused by means of a single focusing lens (41) onto a detector lens (5) so as to produce an image of the sample emissions in the plane of an array of individually addressable photoelectric detectors (CCD (6), fig. 2).
The subject-matter of claim 25 differs therefrom in that
i) a pinhole aperture is located in front of the detector lens,
ii) circuit means are provided to which digitized signals from the array corresponding to the incident light are supplied, and
iii) computing and analysing circuit means as well as memory means for storing data indicative of the emitted light together with address information are included in the apparatus.
Regarding point i) the same argumentation is put forward as with respect to claim 1 above. The circuit means receiving digitized signals (point ii)) as well as computing, analysing and memory means (point iii)) have already been employed for the same purpose in a similar apparatus, see document D2, page 569, right column. Applying these features to a device according to document D1 would be obvious to the person skilled in the art. Therefore, the apparatus according to

claim 25 is not inventive.

Regarding **claim 29** document **D1**, which is considered to represent the most relevant state of the art, discloses a method of analysing fluorescence emitted by radiation excited samples in an array of samples (col. 2, ll. 25-30) comprising the steps of focusing light so as to form a parallel beam (see fig. 2, light paths between micro lenses (2i) and lens (41)), subsequently focusing all parallel light paths through a single point (at lens (5), fig. 2) and re-establishing a parallel array of light beams by the use of a further lens (42, fig. 2) so as to present to a detector array (CCD (6), fig. 2) a plurality of parallel light paths (see fig. 2) and to determine the light incident thereon (implicitly disclosed with a detector).
The subject-matter of claim 29 differs therefrom in that a plate with a small aperture is located at the focusing point of all light paths and in that data relating to the quantity of incident light and address information is stored.
However, a plate with a small aperture and storage means have already been used for the same purpose in a similar method, see document D2, figure 4 (aperture (2)) and page 569, right column. It would be obvious to the person skilled in the art to apply these features to document D1, thereby arriving at a method according to claim 29.

2.2. Dependent **claims 2-24, 26-28 and 30-32**

Dependent **claims 2-24, 26-28 and 30-32** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows:

With respect to **claim 2**, see D1, figs. 1 and 2.
With respect to **claim 3**, see D1, lens (41, fig. 2) and focusing point at lens (5, fig. 2)
With respect to **claim 4**, see D1, figure 1.
With respect to **claim 5**, see D1, figure 2, micro titre plate (1) and lenses (2i).
With respect to **claims 7 and 8**, see D1, col. 3, second paragraph.
With respect to **claims 9-11**, see D1, col. 2, lines 50-59 and figure 2.
With respect to **claim 12**, see D1, col. 2, line 64 and the beam expander (42, 5, 41, fig.2).

With respect to **claim 13**, see D1, col. 1, line 63 - col. 2, line 7 and col. 2, line 65 - col. 3, line 1.
With respect to **claim 14**, see D3, page 22, third paragraph.
With respect to **claim 15**, see D1, col. 2, lines 25-27.
With respect to **claim 16 and 19**, see D3, page 1, third paragraph.
With respect to **claim 18**, see D1, col. 4, ll. 14-20.
With respect to **claim 20**, see D3, page 1, third paragraph and page 10, last paragraph - page 11, first paragraph.
With respect to **claim 22**, see D1, figure 2, micro lenses (21).
With respect to **claim 23**, see D1, figure 2.
With respect to **claim 24**, see D1, col. 4, lines 14-20.
With respect to **claim 26**, see D1, see figure 2: beam splitter (8), parallel beam between micro lenses (2i) and lens (41), and the incident light (col. 2, lines 50-59) and filter means (col. 3, lines 2-7).
With respect to **claim 27**, see D1, laser (col. 2, line 64), beam expander (fig. 2) and beam splitter (92, fig. 2).
With respect to **claims 28 and 30**, see D3, page 10, last paragraph - page 11, first paragraph.
With respect to **claim 31**, see D1, figure 2.
With respect to **claim 32**, see D3, page 10, last paragraph - page 11, first paragraph (shutter) and D2, page 569, right column (signal processing and storage).

The subject-matter of **claims 6, 17 and 21** relates to structural changes which come within the scope of the customary practice followed by persons skilled in the art.

3. The industrial applicability of **claims 1-32** is beyond doubt, Article 33(4) PCT.

Re Item VII

Certain defects in the international application

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 and D3 is not mentioned in the description, nor are

these documents identified therein.

2. The features of the apparatus claims are not provided with reference signs placed in parentheses (Rule 6.2(b) PCT).

Re Item VIII

Certain observations on the international application

1. The expression "upstream and downstream regions" used in **claim 1** is unclear as there is no stream of fluid indicating a flow direction (Article 6 PCT).

2. The term "downstream optical system" used in **claim 2** is not defined in claim 1 and leaves the reader in doubt as to what element the downstream optical system represents, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT). The same argument can be put forward with respect to the term "mask" in **claim 18**.

3. The subject-matter of **claim 19** is formulated as a claim dependent on claim 17 or 18. Yet, claim 19 does not comprise all features of claim 17 or 18 and is therefore not a dependent claim.

4. **Claim 20** lists different detectors which seem to be alternatives. However, the formulation "photomultiplier tubes, image intensifiers **and** intensified CCD arrangements" conveys the impression that all three alternative detectors are used simultaneously. Hence, there is an inconsistency between the way of presenting these (alternative) features.

PATENT COOPERATION TREATY

REC'D 11 SEP 2001

WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference C1124.01/C	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB00/01576	International filing date *(day/month/year)* 20/04/2000	Priority date *(day/month/year)* 26/06/1999
International Patent Classification (IPC) or national classification and IPC G01N21/25		
Applicant PACKARD INSTRUMENT COMPANY, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 17 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/01/2001	07.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Huenges, A Telephone No. +49 89 2399 2280

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01576

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-5,8-10,17-24	as originally filed			
6,7,7a,11-16, 16a	as received on	05/07/2001	with letter of	28/06/2001

Claims, No.:

1-32	as received on	05/07/2001	with letter of	28/06/2001

Drawings, sheets:

1/6-6/6	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-32
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-32
Industrial applicability (IA)	Yes:	Claims	1-32
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: DE 197 48 211 A (CARL ZEISS) 6 May 1999 (1999-05-06)
D2: H.J. TIZIANI ET AL.: 'Three-dimensional analysis by a microlens-array confocal arrangement' APPLIED OPTICS., vol. 33, no. 4, 1 February 1994 (1994-02-01), pages 567-572, XP000429123 OPTICAL SOCIETY OF AMERICA,WASHINGTON., US ISSN: 0003-6935
D3: GB-A-2 315 131 (CAMBRIDGE IMAGING) 21 January 1998 (1998-01-21)

2. **Inventive step**

2.1. Independent **claims 1, 25 and 29**

The subject-matter of **claims 1, 25 and 29** is new (Article 33(2) PCT) but not inventive (Article 33(3) PCT).

Regarding **claim 1** document **D1** discloses a method of imaging a plurality of micro-sample light emitting sites simultaneously onto separately addressable detectors (col. 2, ll. 25-30) wherein a plurality of objective lenses (2i, fig. 2) are located adjacent to the micro-sample array (see micro titre plate (1), fig. 2) with one objective lens for each micro-sample (col. 3, ll. 19-21), the latter are located at or near the focal point of each of the lenses (the position of the micro-sample array in fig. 2 is necessarily at the focal point of the micro-sample array as the light beam between the micro-lens array (2i) and the focussing lens (41) is parallel and as the beam focuses to a single point at the position of the lens (5), fig. 2) and the beams are focused by a focusing lens through a single point (at the position of lens (5), fig. 2) and collected beyond that point by detector lens means (42, fig. 2) which serves to reconstitute the parallel beams (see fig. 2) for presentation to the detector array (6, fig. 2).
The subject-matter of claim 1 differs therefrom in that a pinhole aperture is located at the focusing point of the focussing lens.

The problem to be solved by the present invention may therefore be regarded as the detection of light solely emanating from the focal point of each of the objective lenses.
However, a plate with a small aperture for detection of light emanating from the focal point of each of the objective lenses of a microlens array has already been used in a similar method, see document D2, figure 4 (aperture (2)). It would be obvious to the person skilled in the art, namely when the same result is to be achieved, to apply this feature to document D1, thereby arriving at a method according to claim 29.

Regarding **claim 25**, document **D1**, which is considered to represent the most relevant state of the art, discloses an apparatus adapted for performing the method of claims 1 or 8, comprising means for supporting a micro-sample array on a substrate (means for supporting the micro-titre plate (1, fig. 2) are implicitly disclosed) in close proximity but parallel to an array of micro lenses (2i, fig. 2) arranged so as to correspond on a one to one basis with the positions and spacing of at least some of the micro-samples, the micro-samples being positioned at the focal points of their related micro lenses (implicitly disclosed with parallel rays between micro lenses (2i) and lens (41), fig. 2), and the parallel beams of light are focused by means of a single focusing lens (41) onto a detector lens (5) so as to produce an image of the sample emissions in the plane of an array of individually addressable photoelectric detectors (CCD (6), fig. 2).
The subject-matter of claim 25 differs therefrom in that
i) a pinhole aperture is located in front of the detector lens,
ii) circuit means are provided to which digitized signals from the array corresponding to the incident light are supplied, and
iii) computing and analysing circuit means as well as memory means for storing data indicative of the emitted light together with address information are included in the apparatus.
Regarding point i) the same argumentation is put forward as with respect to claim 1 above. The circuit means receiving digitized signals (point ii)) as well as computing, analysing and memory means (point iii)) have already been employed for the same purpose in a similar apparatus, see document D2, page 569, right column. Applying these features to a device according to document D1 would be obvious to the person skilled in the art. Therefore, the apparatus according to

claim 25 is not inventive.

Regarding **claim 29** document **D1**, which is considered to represent the most relevant state of the art, discloses a method of analysing fluorescence emitted by radiation excited samples in an array of samples (col. 2, ll. 25-30) comprising the steps of focusing light so as to form a parallel beam (see fig. 2, light paths between micro lenses (2i) and lens (41)), subsequently focusing all parallel light paths through a single point (at lens (5), fig. 2) and re-establishing a parallel array of light beams by the use of a further lens (42, fig. 2) so as to present to a detector array (CCD (6), fig. 2) a plurality of parallel light paths (see fig. 2) and to determine the light incident thereon (implicitly disclosed with a detector).
The subject-matter of claim 29 differs therefrom in that a plate with a small aperture is located at the focusing point of all light paths and in that data relating to the quantity of incident light and address information is stored.
However, a plate with a small aperture and storage means have already been used for the same purpose in a similar method, see document D2, figure 4 (aperture (2)) and page 569, right column. It would be obvious to the person skilled in the art to apply these features to document D1, thereby arriving at a method according to claim 29.

2.2. Dependent **claims 2-24, 26-28 and 30-32**

Dependent **claims 2-24, 26-28 and 30-32** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows:

With respect to **claim 2**, see D1, figs. 1 and 2.
With respect to **claim 3**, see D1, lens (41, fig. 2) and focusing point at lens (5, fig. 2)
With respect to **claim 4**, see D1, figure 1.
With respect to **claim 5**, see D1, figure 2, micro titre plate (1) and lenses (2i).
With respect to **claims 7 and 8**, see D1, col. 3, second paragraph.
With respect to **claims 9-11**, see D1, col. 2, lines 50-59 and figure 2.
With respect to **claim 12**, see D1, col. 2, line 64 and the beam expander (42, 5, 41, fig.2).

With respect to **claim 13**, see D1, col. 1, line 63 - col. 2, line 7 and col. 2, line 65 - col. 3, line 1.
With respect to **claim 14**, see D3, page 22, third paragraph.
With respect to **claim 15**, see D1, col. 2, lines 25-27.
With respect to **claim 16 and 19**, see D3, page 1, third paragraph.
With respect to **claim 18**, see D1, col. 4, ll. 14-20.
With respect to **claim 20**, see D3, page 1, third paragraph and page 10, last paragraph - page 11, first paragraph.
With respect to **claim 22**, see D1, figure 2, micro lenses (21).
With respect to **claim 23**, see D1, figure 2.
With respect to **claim 24**, see D1, col. 4, lines 14-20.
With respect to **claim 26**, see D1, see figure 2: beam splitter (8), parallel beam between micro lenses (2i) and lens (41), and the incident light (col. 2, lines 50-59) and filter means (col. 3, lines 2-7).
With respect to **claim 27**, see D1, laser (col. 2, line 64), beam expander (fig. 2) and beam splitter (92, fig. 2).
With respect to **claims 28 and 30**, see D3, page 10, last paragraph - page 11, first paragraph.
With respect to **claim 31**, see D1, figure 2.
With respect to **claim 32**, see D3, page 10, last paragraph - page 11, first paragraph (shutter) and D2, page 569, right column (signal processing and storage).

The subject-matter of **claims 6, 17 and 21** relates to structural changes which come within the scope of the customary practice followed by persons skilled in the art.

3. The industrial applicability of **claims 1-32** is beyond doubt, Article 33(4) PCT.

Re Item VII

Certain defects in the international application

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 and D3 is not mentioned in the description, nor are

these documents identified therein.

2. The features of the apparatus claims are not provided with reference signs placed in parentheses (Rule 6.2(b) PCT).

Re Item VIII

Certain observations on the international application

1. The expression "upstream and downstream regions" used in **claim 1** is unclear as there is no stream of fluid indicating a flow direction (Article 6 PCT).

2. The term "downstream optical system" used in **claim 2** is not defined in claim 1 and leaves the reader in doubt as to what element the downstream optical system represents, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT). The same argument can be put forward with respect to the term "mask" in **claim 18**.

3. The subject-matter of **claim 19** is formulated as a claim dependent on claim 17 or 18. Yet, claim 19 does not comprise all features of claim 17 or 18 and is therefore not a dependent claim.

4. **Claim 20** lists different detectors which seem to be alternatives. However, the formulation "photomultiplier tubes, image intensifiers **and** intensified CCD arrangements" conveys the impression that all three alternative detectors are used simultaneously. Hence, there is an inconsistency between the way of presenting these (alternative) features.

INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/GB 00/01576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/25 G01N21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, INSPEC, COMPENDEX, IBM-TDB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 197 48 211 A (CARL ZEISS) 6 May 1999 (1999-05-06) abstract page 1, line 63 -page 2, line 16 page 2, line 25 - line 30 page 2, line 64 page 3, line 2 - line 7 page 3, line 19 - line 21	1-3,8, 10,12, 13,15, 16,21, 24,31
Y	figures	4,5,11, 17-20, 22,23, 25, 30-32, 34,37,39

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 2000	17/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Thomas, R.M.

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 00/01576

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	H.J. TIZIANI ET AL.: "Three-dimensional analysis by a microlens-array confocal arrangement" APPLIED OPTICS., vol. 33, no. 4, 1 February 1994 (1994-02-01), pages 567-572, XP000429123 OPTICAL SOCIETY OF AMERICA,WASHINGTON., US ISSN: 0003-6935	11, 17-20, 32,34, 37,39
A	page 569, right-hand column; figure 4	36
Y	GB 2 315 131 A (CAMBRIDGE IMAGING) 21 January 1998 (1998-01-21) page 1, paragraph 1 - paragraph 3 page 10, last paragraph -page 11, line 1 page 18, paragraph 3 - paragraph 4	4,5,22, 23,25, 30,31
A	figure 1	27,28, 38,42
A	WO 97 34171 A (JOHNSON) 18 September 1997 (1997-09-18) page 5, line 21 -page 6, line 13 page 29, line 6 - line 9 page 37, line 32 -page 38, line 10 figures 1,36	6,7,9,14
A	WO 98 30889 A (MEDISPECTRA) 16 July 1998 (1998-07-16) page 14, line 5 -page 15, line 6 figure 3	38,42

1

INTERNATIONAL SEARCH REPORT

..ormation on patent family members

Inten nal Application No
PCT/GB 00/01576

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 19748211 A	06-05-1999	AU 9749898 A	24-05-1999
		WO 9923474 A	14-05-1999
GB 2315131 A	21-01-1998	EP 0910790 A	28-04-1999
		EP 0910791 A	28-04-1999
		WO 9801743 A	15-01-1998
		WO 9801744 A	15-01-1998
		GB 2315130 A	21-01-1998
WO 9734171 A	18-09-1997	AU 1975197 A	01-10-1997
		EP 0991959 A	12-04-2000
WO 9830889 A	16-07-1998	EP 0951643 A	27-10-1999

Internat[redacted] Application No
PCT/[redacted] 00/01576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/25 G01N21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, INSPEC, COMPENDEX, IBM-TDB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 197 48 211 A (CARL ZEISS) 6 May 1999 (1999-05-06) abstract page 1, line 63 -page 2, line 16 page 2, line 25 - line 30 page 2, line 64 page 3, line 2 - line 7 page 3, line 19 - line 21	1-3,8, 10,12, 13,15, 16,21, 24,31
Y	figures	4,5,11, 17-20, 22,23, 25, 30-32, 34,37,39
	--- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 2000	17/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Thomas, R.M.

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	H.J. TIZIANI ET AL.: "Three-dimensional analysis by a microlens-array confocal arrangement" APPLIED OPTICS., vol. 33, no. 4, 1 February 1994 (1994-02-01), pages 567-572, XP000429123 OPTICAL SOCIETY OF AMERICA,WASHINGTON., US ISSN: 0003-6935	11, 17-20, 32,34, 37,39
A	page 569, right-hand column; figure 4	36
Y	GB 2 315 131 A (CAMBRIDGE IMAGING) 21 January 1998 (1998-01-21) page 1, paragraph 1 - paragraph 3 page 10, last paragraph -page 11, line 1 page 18, paragraph 3 - paragraph 4	4,5,22, 23,25, 30,31
A	figure 1	27,28, 38,42
A	WO 97 34171 A (JOHNSON) 18 September 1997 (1997-09-18) page 5, line 21 -page 6, line 13 page 29, line 6 - line 9 page 37, line 32 -page 38, line 10 figures 1,36	6,7,9,14
A	WO 98 30889 A (MEDISPECTRA) 16 July 1998 (1998-07-16) page 14, line 5 -page 15, line 6 figure 3	38,42

1

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 19748211 A	06-05-1999	AU 9749898 A	24-05-1999
		WO 9923474 A	14-05-1999
GB 2315131 A	21-01-1998	EP 0910790 A	28-04-1999
		EP 0910791 A	28-04-1999
		WO 9801743 A	15-01-1998
		WO 9801744 A	15-01-1998
		GB 2315130 A	21-01-1998
WO 9734171 A	18-09-1997	AU 1975197 A	01-10-1997
		EP 0991959 A	12-04-2000
WO 9830889 A	16-07-1998	EP 0951643 A	27-10-1999

Thus a single channel confocal scanner has been used to measure a number of sample sites but only sequentially and not simultaneously, and such a system is not appropriate for high throughput scanning where simultaneous detection and assessment of a large number of sites is required, such as in a 96 well microtitre plate or higher density format array or array of microcapillaries.

However, there is known from German Specification No DE 19748211A a system in which a plurality (N) of single channel confocal optical systems and photoelectric detectors or detecting areas are arranged in parallel to form a plurality of reading heads arranged side-by-side so as simultaneously to read a corresponding plurality of adjacent sites. In this known system, the optics include a mask and three focussing lenses which effectively handle the beams emanating from the individual reading heads independently, so that the optical axes of the individual beams are separated.

Summary of the invention

According to one aspect of the present invention, there is provided a method of imaging a plurality of micro-sample light emitting sites simultaneously onto separate addressable detectors, which may be discrete regions of the array detector, so that light emitted from each site can be monitored by one of the detectors, wherein a corresponding plurality of objective lenses each comprising a micro-lens are located adjacent to the micro-sample array with one objective lens for each micro-sample, the latter are located at or near the focal point of each of the micro-lenses so that light emanating from each micro-sample is collected by

its respective objective lens and converted into a beam of parallel or near parallel rays, the objective lenses are arranged so that the axes of all the beams issuing therefrom are parallel and spaced apart, and the beams are focused by a focussing lens through single point and collected beyond that point by detector lens means which serves to reconstitute the parallel beams for presentation to the detector array, wherein optical discrimination between fluorescence emanating from upstream and downstream regions of the micro-samples is improved by a pinhole aperture located at the focal point of the focussing lens so that light which is not emanating from the focal point of each of the objective lenses adjacent the micro-sample sites will be out of focus at the small aperture pinhole.

The sites may be in a column or in an array such as a microtitre plate.

The optical systems may be arranged in a single line for reading column by column of a multi-column array, or in a staggered pattern for simultaneously viewing sites in different columns if this were preferred.

This system would again comprise essentially (N) independent confocal systems, each with its optic axis aligned with one sample site.

Advantageously only one laser source is provided which may be split into an appropriate plurality of beams, conveyed each by a fibre optic cable to individual sample sites.

The light emitted from the separate sites may in part be conveyed to individual detectors, or discrete regions of an array detector, via optical fibres.

Preferably the reading heads are independently adjustable so each can be positioned accurately over or under a sample. To this end a special opto-mechanical device may be provided to bring each scanner's optic axis into alignment by means of an appropriate scan in the Y-direction.

Where there is a tolerance in the position of the samples, such as in the case of capillaries, which result in uncertainty in the Y-direction, adjustment is preferably provided.

Such an arrangement could be used to inspect say 8 or 12 sites at a time, but an arrangement to inspect larger numbers simultaneously could be costly, complex, and cumbersome. We would refer to such arrangements as "ganged" systems.

The axes of the objectives may be angled if desired with

relative to the array of objective lenses in manner known per se so as to present groups of 96 wells making up the 384 wells, to the 96 lens array. Thus a 2 x 2 movement of the XY stage would be required to complete the inspection of all of the well sites in the 384 well array.

According to another optional feature of the invention, the parallel beams of light directed towards the detector array may in part be transferred to the latter via optical fibres, preferably in the form of a fibre optic bundle or fibre optic plate.

According to a further preferred feature of the invention, where a bundle of fibres is employed, the arrangement of the fibres in the bundle may differ between the input and output ends thereof so that the array of fibres which convey the light paths to the different regions of the detector array conforms more to the shape of the detector array in the XY plane and enables for example, optimal utilisation of a generally square array by the rectangular proportions of an 8 x 12 micro-sample array.

The detector array may typically be a charge coupled device having a large number of separately addressable regions (each of which is commonly referred to as a pixel) and groups of adjacent pixels (or individual pixels) from the detector for each sample are addressed, to enable good resolution to be obtained in the XY sense as between the light from one sample and another.

The charge coupled device may be cooled, for example cryogenically so as to reduce background noise signals associated therewith.

According to a further feature of the invention, the detectors comprise an array of photomultipliers, one photomultiplier for each of the channels (optical paths).

Thus if there are 96 channels, 96 photomultipliers will be required.

Preferably miniature photomultipliers are employed, and where sufficiently small photomultipliers are unavailable, optical fibres or bundles forming cables may be employed to convey the light from each of the apertures in the mask such as is shown in Figure 4 or 5 of the accompanying drawings, to the 96 windows of 96 photomultipliers which together occupy an area considerably greater than that of the mask.

Alternatively a larger detector lens is employed so that the 96 channels are spread over a larger area.

As previously mentioned, the CCD array may of course be cryogenically or otherwise cooled for the sample purpose, the use of photomultipliers obviates the need for cryogenic cooling.

According to another feature of the invention, the photomultipliers are replaced by an image intensifier or an intensified CCD.

The use of a more sensitive detector such as the use of photomultiplier tubes, an image intensifier or an intensifier CCD, enables time resolved fluorescence of luminescence application to be detected.

In particular the use of a more sensitive detector enables time resolved assay analysis to be performed which involves photo-exciting a fluorophor with a pulsed source of radiation for example a pulsed laser, during which time the detector is gated off so as not to be responsive to any of the excitation radiation and the fluorophor in the assay can be investigated by gating on the detector after a suitable time delay, typically of the order of picoseconds or microseconds, and thereafter reading out the charge pattern in the detector.

Time resolved applications such as described require detectors which can be gated on and off and photomultiplier tubes, image intensifiers and intensified CCD arrangements can be gated in this way electronically so as to enable the delays and short integration periods to be generated as required by time resolved fluorescence or luminescence applications.

In this connection, it is to be understood that the present invention is not limited to fluorescence applications but includes luminescence applications.

Thus the invention is not limited to the nature of the detector or to the technique in which the different regions of the detector are addressed to enable light emanating from one sample to be distinguished from light emanating from another, nor is the invention limited to any of the circuits or computing techniques which may be employed for processing electrical signals obtained by addressing the different regions of the detector array.

Since the spacing between, and the actual size of, the sample sites in a micro-sample array, are very small, and can be of the order of microns, micro lenses, optionally in combination with a fibre optic transfer plate may be employed in the objective lenses adjacent the micro-samples.

In essence the micro-sample objective lenses are equivalent to microscope objectives in that they are short focal length lenses designed to form an image of a tiny object (in this case light being emitted from a small region in a micro-sample). However as distinct from a conventional microscope objective, the optical characteristic of the sample objectives is such as to produce a parallel beam of light therefrom so that in this sense the objective can be said to have one infinite conjugate.

By utilising high quality components and projecting the excitation radiation to the samples via the objective lenses, the excitation radiation can be focused to precisely the regions of interest in the samples, to reduce excitation of surrounding sample material.

Where a filter is located ahead of the detector, apertured masks may be placed on either side of the filter to collimate the parallel beam to further reduce background and cross-talk.

The invention also lies in apparatus adapted to perform the above methods and comprising means for supporting a micro-sample array on a substrate in close proximity but parallel to an array of micro lenses arranged so as to correspond on a one to one basis with the positions and spacing of at least some of the micro-samples on the substrate, each of

the micro lenses being positioned relative to a region of its related micro-sample by a distance equal to the focal length of the lens so that light emitted from that region of the micro-sample will emerge from the lens as a parallel beam parallel to the axis of the lens, and the parallel beams of light are focused by means of a single focusing lens through a pinhole aperture onto a detector lens so as to produce an image of the micro-sample light emissions in the plane of an array of individually addressable photoelectric detectors, such as regions of an addressable CCD array, and circuit means is provided to which signals read out from the array are supplied in the form of a sequence of digital values or otherwise, each corresponding to the light incident on a region of the detector for a given period of time from one of the micro-samples, and computing and analysing circuit means is provided, responsive to the electrical signals, together with memory means for storing data indicative of the light found to be emitted from each of the micro-samples, for storing those values together with address information, whereby each stored value can be identified with the micro-sample on the substrate from which the light giving that value has been emitted by reference to the position of the region in the detector array and by correlating the position of the sample in the sample array.

The apparatus may further include a beam splitter, such as a dichroic mirror, interposed in the optical path between the micro lenses and the focusing lens to enable on the one hand light to pass from the lenses to the focusing lens, and on the other hand to enable excitation radiation, typically light of a particular wavelength, to be reflected as a parallel beam towards the micro lenses, thereby utilising

the optical focusing characteristics of the micro lenses to focus the parallel light into spots of light which register with the micro-samples so that the latter are individually radiated by excitation light which is predominantly incident on that region of each micro-sample which is to be inspected for fluorescence after the excitation radiation has been removed, and filter means may be provided in the optical path between the beam splitter and the detector array to generally attenuate any excitation wavelength radiation travelling towards the detector and generally prevent such radiation from reaching the detector.

The apparatus preferably includes a pinhole aperture at the focal point of the focusing lens so as to improve the on-axis resolution of the optical system and assist in attenuating unwanted fluorescence such as from background material and other components of an assay ahead of or behind the region of interest in a micro-sample measured along the optical axis of the objective, from reaching the detectors.

The apparatus typically includes a laser source, as the source of excitation radiation a beam expander for enlarging the cross-section of the laser beam and presenting a generally uniform parallel beam of excitation radiation for entry into the imaging system via the beam splitter or dichroic mirror.

Shutter means may be provided to inhibit the passage of the laser light, except when required for excitation purposes, and further shutter means may be provided synchronised with that associated with the laser source to prevent light of any wavelength reaching the detector whilst excitation light is projected into the system.

The invention also comprises a method of analysing fluorescence emitted by radiation excited samples in an array of samples comprising the steps of focusing light emitted from each said sample at infinity so as to form a parallel beam, in parallel with the light from all of the other sample sites making up the array, subsequently focusing all the parallel light paths through a single point and locating at the point a small pinhole aperture to restrict unwanted light from fluorescing material upstream and downstream of the sites of interest in the samples, and re-establishing a parallel array of light beams by the use of a further lens so as to present to an addressable detector array a plurality of parallel light paths corresponding to the light paths from the samples, and individually addressing different regions of the detector array onto which the parallel light paths impinge, to determine the light incident thereon, and storing data relating to the quantity of incident light on each said region of the detector array together with address information to enable the data to be reconciled with the position of the sample in the array on the substrate to which that data relates.

The method typically further comprises the step of introducing periodically into the optical system excitation wavelength illumination and projecting same through the optical imaging devices associated with the array of samples to project the excitation illumination onto a specific region in each said sample, thereafter extinguishing the excitation wavelength light and enabling fluorescence caused by the excitation to

CLAIMS

1. A method of imaging a plurality of micro-sample light emitting sites simultaneously onto separately addressable detectors, which may be discrete regions of the array detector, so that light emitted from each site can be monitored by one of the detectors, wherein a corresponding plurality of objective lenses each comprising a micro-lens are located adjacent to the micro-sample array with one objective lens for each micro-sample, the latter are located at or near the focal point of each of the micro-lenses so that light emanating from each micro-sample is collected by its respective objective lens and converted into a beam of parallel or near parallel rays, the objective lenses are arranged so that the axes of all the beams issuing therefrom are parallel and spaced apart, and the beams are focused by a focusing lens through a single point and collected beyond that point by detector lens means which serves to reconstitute the parallel beams for presentation to the detector array, wherein optical discrimination between fluorescence emanating from upstream and downstream regions of the micro-samples is improved by a pinhole aperture located at the focal point of the focusing lens so that light which is not emanating from the focal point of each of the objective lenses adjacent the micro-sample sites will be out of focus at the small aperture pinhole.

2. A method according to claim 1, wherein the axes of the objectives are angled with appropriate adjustment of the optical characteristics of the objectives and/or downstream optical system.

3. A method according to claim 1 or claim 2, wherein a single focusing lens (which may be a multi-component lens) is employed for directing all the beam paths through the single point.

4. A method according to any of claims 1 to 3, wherein the micro-samples are positioned relative to the micro-sample objective lenses so that the region of interest is as close as possible to the focal point of the respective objective lens.

5. A method according to any of claims 1 to 4, wherein the samples are located on a planar support with the regions of interest all in the same plane so that the objective lenses can likewise all be in the same plane parallel to that containing the regions of interest in the samples.

6. A method according to any of claims 1 to 5, including the step of adjusting the position of the micro-sample array relative to the lens array and also the step of individually adjusting the position of at least the objective lenses relative to the micro-samples or vice versa to ensure that the regions of interest in the micro-samples are at the focal point of the respective micro-sample objective lenses.

7. A method according to any of claims 1 to 6, wherein in order to provide spectral separation based on wavelength, a filter is included in the light path either between the micro-sample objective lenses and the focusing lens means ahead of the pinhole, or between the detector lens and the detector array.

8. A method according to claim 7, wherein the spectral filter is located in a region in which the light paths are parallel or nearly parallel.

9. A method according to any of claims 1 to 8, according to which where fluorescence is the mechanism which generates the radiation which is to be focused onto a detector, excitation radiation to produce the fluorescence is applied only to a region of interest within each micro-sample rather than over the whole of the micro-sample.

10. A method according to claim 9, wherein excitation radiation is injected into the multipath optical system so as to proceed in a parallel sense towards the array of objective lenses, in an opposite sense to the light which emanates from the micro-

samples, so as to be focused by the objective lenses onto the region of interest in each micro-sample.

11. A method according to claim 10, wherein the excitation radiation is injected as a parallel beam into the optical path, at right angles thereto, and a 45° beam splitting device is provided onto which the parallel excitation radiation is incident and from which it is directed in a parallel manner towards the micro-sample imaging lenses, but through which radiation from the micro-samples can pass to the focusing lens.

12. A method according to any of claims 1 to 11, wherein excitation radiation is produced using a laser such as an argon ion laser, and a beam expander is employed to expand the cross-section of the laser beam into a relatively large area beam equivalent to the area of the parallel array of micro-sample objective lenses.

13. A method according to any of claims 1 to 12, having an 8 x 12 array of micro-sample objective lenses on the same 8 x 12 matrix as a standard 96 well plate, and if the imaging system is to be used to inspect for example a 384 well plate, the latter is mounted on an XY stage so that it can be moved relative to the array of objective lenses in manner known per se so as to present groups of 96 wells making up the 384 wells, to the 96 lens array.

14. A method according to any of claims 1 to 13, wherein the parallel beams of light directed towards the detector array are transferred to the said array via optical fibres, in the form of a fibre optic bundle or fibre optic plate.

15. A method according to any of claims 1 to 14, wherein the detector array is a charge coupled device having a large number of separately addressable regions (each of which is commonly referred to as a pixel) and groups of adjacent pixels (or individual pixels) from the detector for each sample are addressed, to enable good resolution to be obtained in the XY sense as between the light from one sample and another.

~~16. A method according to claim 15, wherein the charge coupled device is cooled, for~~ example cryogenically.

17. A method according to any of claims 1 to 14, wherein the detector array comprises an array of photomultipliers, one photomultiplier for each of the channels (optical paths).

18. A method according to claim 17, wherein optical fibres or bundles forming cables are employed to convey the light from each of the apertures in a mask to the windows of photo-multipliers which together occupy an area considerably greater than that of the mask.

19. A method according to claim 17 or 18, wherein the photomultipliers are replaced by an image intensifier or an intensified CCD.

20. A method according to any of claims 17 to 19, wherein the photomultiplier tubes, image intensifiers and intensified CCD arrangements are gated electronically so as to enable the delays and short integration periods to be generated as required by time resolved fluorescence or luminescence applications.

21. A method according to any of claims 1 to 20, wherein further lenses for focusing the parallel beams of light directed towards the detector array are employed to improve resolution at the detector surface either in combination with a fibre optic transfer bundle, or otherwise.

22. A method according to any of claims 1 to 21, wherein micro lenses, optionally in combination with a fibre optic transfer plate are employed in the objective lenses adjacent the micro-samples.

23. A method according to claim 22, in which micro lenses have one infinite conjugate.

24. A method according to any of claims 1 to 23, wherein where a filter is located ahead of the detector, apertured masks are placed on either side of the filter to collimate the parallel beam to further reduce background and cross-talk.

25. Apparatus adapted for performing the method of any of claims 1 to 24, comprising means for supporting a micro-sample array on a substrate in close proximity but parallel to an array of micro lenses arranged so as to correspond on a one to one basis with the positions and spacing of at least some of the micro-samples on the substrate, each of the micro lenses being positioned relative to a region of its related micro-sample by a distance equal to the focal length of the lens so that light emitted from that region of the micro-sample will emerge from the lens as a parallel beam parallel to the axis of the lens, and the parallel beams of light are focused by means of a single focusing lens through a pinhole aperture onto a detector lens so as to produce an image of the micro-sample light emissions in the plane of an array of individually addressable photoelectric detectors, such as regions of an addressable CCD array, and circuit means is provided to which signals read out from the array are supplied in the form of a sequence of digital values or otherwise, each corresponding to the light incident on a region of the detector for a given period of time from one of the micro-samples, and computing and analysing circuit means is provided, responsive to the electrical signals, together with memory means for storing data indicative of the light found to be emitted from each of the micro-samples, for storing those values together with address information, whereby each stored value can be identified with the micro-sample on the substrate from which the light giving that value has been emitted by reference to the position of the region in the detector array and by correlating the position of the sample in the sample array.

26. Apparatus according to claim 25, further including a beam splitter, such as a dichroic mirror, interposed in the optical path between the micro lenses and the focusing lens to enable on the one hand light to pass from the lenses to the focusing lens, and on the other hand to enable excitation radiation, typically light of a particular wavelength, to be reflected as a parallel beam towards the micro lenses, thereby utilising the optical focusing characteristics of the micro lenses to focus the parallel light into spots of light

which register with the micro-samples so that the latter are individually radiated by excitation light which is predominantly incident on that region of each micro-sample which is to be inspected for fluorescence after the excitation radiation has been removed, and filter means provided in the optical path between the beam splitter and the detector array to generally attenuate any excitation wavelength radiation travelling towards the detector and generally prevent such radiation from reaching the detector.

27. Apparatus according to claim 25 or claim 26, including a laser source as the source of excitation radiation a beam expander for enlarging the cross-section of the laser beam and presenting a generally uniform parallel beam of excitation radiation for entry into the imaging system via the beam splitter or dichroic mirror.

28. Apparatus according to any of claims 25 to 27, including shutter means to inhibit the passage of the source light, except when required for excitation purposes, and further shutter means synchronised with that associated with the source to prevent light of any wavelength reaching the detector whilst excitation light is projected into the system.

29. A method of analysing fluorescence emitted by radiation excited samples in an array of samples comprising the steps of focusing light emitted from each said sample at infinity so as to form a parallel beam, in parallel with the light from all of the other sample sites making up the array, subsequently focusing all the parallel light paths through a single point and locating at the point a small pinhole aperture to restrict unwanted light from fluorescing material upstream and downstream of the sites of interest in the samples, and re-establishing a parallel array of light beams by the use of a further lens so as to present to an addressable detector array a plurality of parallel light paths corresponding to the light paths from the samples, and individually addressing different regions of the detector array onto which the parallel light paths impinge, to determine the light incident thereon, and storing data relating to the quantity of incident light on each said region of the detector array together with address information to enable the data to be reconciled with the position of the sample in the array on the substrate to which that data relates.

30. The method according to claim 29, further comprising the step of introducing periodically into the optical system excitation wavelength illumination and projecting same through the optical imaging devices associated with the array of samples to project the excitation illumination onto a specific region in each said sample, thereafter extinguishing the excitation wavelength light and enabling fluorescence caused by the excitation to pass through the same optical devices to emerge as parallel rays of light for transfer to a detector, for analysis as above mentioned.

31. The method according to claim 29 or claim 30, wherein the objectives are arranged above or below a sample array.

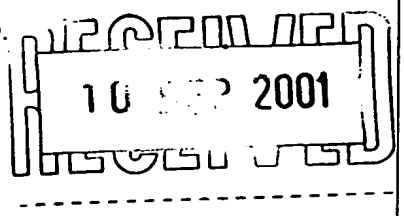
32. The method according to any of claims 29 to 31, when used to perform immediate fluorescence analysis or time resolved fluorescence analysis in which a shutter is provided to inhibit the transfer of light to the detector, excitation radiation is supplied for a short interval of time and then shut off (either by pulsing the source out using further shutter or both), after a selected interval of time the shutter preventing transfer of light to the detector is opened and after an appropriate integration interval (which can be very short or longer as described) the residual charge pattern on the charge coupled detector array is interrogated to generate a signal relating to the charge pattern (and therefore indicative of light incident thereon) for processing and storage as aforesaid.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

KEITH W. NASH & CO.
90-92 Regent Street
Cambridge CB2 1DP
GRANDE BRETAGNE



PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	07.09.2001
Applicant's or agent's file reference C1124.01/C	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01576	20/04/2000	26/06/1999

Applicant
PACKARD INSTRUMENT COMPANY, INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Choulouilidou, C Tel.+49 89 2399-2257

Form PCT/IPEA/416 (July 1992)

PA[illegible]T COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

KEITH W NASH & CO.
90-92 Regent Street
Cambridge CB2 1DP
ROYAUME-UNI

Date of mailing (day/month/year)
14 March 2001 (14.03.01)

Applicant's or agent's file reference
KWN/C1124.01/C

IMPORTANT NOTIFICATION

International application No.
PCT/GB00/01576

International filing date (day/month/year)
20 April 2000 (20.04.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
CAMBRIDGE IMAGING LIMITED
St Johns Innovation Centre
Cowley Road
Cambridge CB4 4WS
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address
PACKARD INSTRUMENT COMPANY, INC.
800 Research Parkway
Meriden, CT 06450
United States of America

State of Nationality: US
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
I. Britel
Telephone No.: (41-22) 338.83.38

003894130

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 19 February 2001 (19.02.01)	
International application No. PCT/GB00/01576	**Applicant's or agent's file reference** KWN/C1124.01/C
International filing date (day/month/year) 20 April 2000 (20.04.00)	**Priority date** (day/month/year) 26 June 1999 (26.06.99)
Applicant RUSHBOOKE, John, Gordon et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 January 2001 (15.01.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Pascal Piriou Telephone No.: (41-22) 338.83.38

GB0001576

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